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INCREASE IN RANGE OF TEMPERATURE TOLERANCE BY ACCLIMATION IN THE COPEPOD *EURYTEMORA AFFINIS*

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Adaptation to temperature change is an obvious requirement for the survival of a temperate species whose habitat is subject to seasonalities. The response of a population to environmental stress depends on the time and intensity of the stress. Slobodkin and Rapoport (1974) suggest that if one level of response (for example physiological) is sufficient to meet the environmental challenge, the next level (for example change in gene frequency) need not be invoked.

The calanoid copepod, *Eurytemora affinis* (Poppe), found in the Chesapeake Bay in temperatures ranging from 0 to 30° C, has many generations per year; hence, it could adapt to this variability in temperature either on an individual or population level. If the range of individual tolerance were sufficient, the species might not need to change genetically through the year. Bradley (1975) found that individual copepods could tolerate the 0–30° C range for short periods.

The question addressed in this paper is whether individuals can become adapted physiologically to a wider range of temperatures (acclimation). This paper also explores the effects of temperature, salinity, sex and stage of development on acclimation to high temperature, the effects of temperature and sex on acclimation to low temperature, and the relationship between tolerances to high and low temperature.

Materials and Methods

Specimens of *Eurytemora* used in some of the experiments were descended from animals collected from Bear Creek in the upper Chesapeake Bay in winter. In other experiments, on salinity effects and on survival, the animals originated in the middle reaches of the Patuxent River, Maryland, in late spring. The reason for

the different collection sites was the availability of specimens. Only one source of animals was used for each experiment. The experiments on heat tolerance, except those on salinity effects, were done in water from the Patuxent River with near zero salinity. Those on cold tolerance were done in 5‰ water from Bear Creek. Salinity was measured with a refractometer.

Acclimation is defined in this paper as the increase in temperature tolerance of individuals following exposure to a temperature closer to the extreme temperature, whether high or low.

Tolerance to high temperature was measured using the shock-recovery assay (Bradley, 1975), permitting data to be obtained on individual copepods. The measurements were highly repeatable with test-retest correlations of around 0.8, and were also closely related to survival time at high temperatures (Bradley, 1976). Single animals in 2 ml of water in test-tubes were immersed in an aquarium held constant at 34.5° C using a heating-stirring unit. No temperature gradients in the aquarium were detected, and the temperature in the test-tubes reached 34.5° C within 90 seconds. Time to succumb (TS), or enter a coma, and time to recover (TR) were observed during a 30 min exposure of all test animals to 34.5° C. Animals were considered comatose when rotating and agitating the vial failed to rouse them. Recovery was noted at the first movement. The assay period was 30 min, and the measures of tolerance were combined in an index $30 + TS - TR$, which could range from 0 to 60, the higher number indicating the greatest tolerance. All animals were exposed to the 34.5° C temperature for 30 min, and those failing to recover while exposed to this temperature were given a TR of $30 - TS$, the index becoming 2TS in these cases. No attempts were made to distinguish between coma and lethality, but both TS alone and the index $(30 + TS - TR)$ were closely related to survival time at 30° C and higher (Bradley, 1976).

Similar methods were used to test tolerance to low temperatures. In the latter case, animals were placed in an aquarium at 0.5° C, and all were removed after 10 min (whether succumbed or not). A majority of animals did become comatose before 10 min, and recovery could be more easily observed at room temperature. Animals not succumbing at all were arbitrarily scored 50, the remainder using the index described above. The next largest score was 40 (animals succumbing at 10 min, recovering immediately), since animals became comatose at or before 10 min or not at all, so setting the maximum tolerance at 50 rather than 60 reduced the discontinuity of tolerance scores.

In the present study 12 animals and all treatment groups were included in each run. Each set of experiments was done by the same observer. In the case of heat tolerance, variance between replicate runs was treated as error variance, since no interactions between treatment and run were detected. The net effect of ignoring runs was to make the tests of variables more conservative because of the increased error variance. In the case of cold tolerance, differences between runs were quite large, due to difficulties in controlling the low temperature at exactly 0.5° C. So run variance (and interaction) were included in the analyses of variances of cold tolerances.

Tolerance was also observed as longevity in two constant temperatures and in a

slowly increasing temperature. Acclimation was observed in these cases as the increase in duration of activity in the test temperature regime of animals previously exposed to an intermediate temperature. Half the animals tested in increasing temperatures were maintained at 24° C for 24 hr before the temperature was raised from 24° C to 31° C in 30 min. The remainder were kept at 15° C. Only males were included in these experiments in increasing temperatures. All the animals were then exposed to a temperature of 31° C initially, which was increased 1° C every 30 min. The test animals were continuously monitored and the times when each animal succumbed and could not be roused were noted. In this case, the end-

TABLE I

Increased temperature tolerance of animals raised at 10° C and exposed to 18° C and 24° C for three periods of time. Body of table gives mean tolerances (in min) to high temperature measured by the shock-recovery assay described in Methods.

Mean temperature tolerances

		Time of exposure		
		2 day	4 day	7 day
Exposure temperature				
10° C (control)	♀	7.5	9.3	5.8
	♂	5.8	7.0	5.8
18°C	♀	14.0	12.8	7.3
	♂	9.0	8.8	10.8
24° C	♀	29.0	40.8	36.6
	♂	21.0	22.8	16.5
		8 animals per mean, 144 total		

Variance analyses for each sex

	Mean squares	
	Females	Males
Time of exposure		
Days at 10° C	24.5	8.3
18° C	63.2	19.0
24° C	284.3	166.4*
Temperature of exposure		
18° C, 24° C *vs.* 10° C	3792.5*	1190.3*
18° C *vs.* 24° C	7575.2*	1344.1*
Within subclass	159.7	21.0
Total variances	312.3	57.1

* $P < 0.01$.

TABLE II

Increased high temperature tolerances of males raised at 20° C compared to 10° C and exposed to three temperatures for two days. Body of table gives tolerances measured by the shock-recovery assay. All animals recovered when raised at 20° C and two of 24 recovered when raised at 10° C.

Mean temperature tolerance		
	Raised at 10° C	Raised at 20° C
Exposure temperature		
10° C	6.7	31.7
18° C	10.0	43.4
24° C	25.0	47.3
	8 animals per mean	9 animals per mean

Variance analyses		
	Mean squares	
	Raised at 10° C	Raised at 20° C
Exposure temperature		
18° C, 24° C *vs.* 10° C	622.0*	1117.9*
18° C *vs.* 24° C	900.0*	68.4
Within subclass	39.4	102.5
Total variances	102.2	140.7

* $P < 0.01$.

point may not have been death itself, but tantamount to death, since recovery did not occur in the increasing temperature.

The relationships between heat and cold tolerance were measured as correlations between observations on the same animals assayed for cold tolerance and heat tolerance 5–6 hr apart on one day. Both assays were repeated the next day, thus two assays for heat tolerance and two for cold tolerance were done on each animal.

When the data in each of the experiments were analyzed, the sexes were treated separately. This was done because of the observed differences between the means and variances of temperature tolerances of the two sexes.

RESULTS

Acclimation to increased temperature occurs in *Eurytemora affinis* (Table I). The set of animals exposed to 18° C or 24° C prior to testing were significantly more tolerant than those kept at 10° C, their rearing temperature. The largest effect was clearly in animals exposed to 24° C, since they were significantly more tolerant than those exposed to 18° C. Time of exposure had a relatively small effect, although it was significant in males exposed to 24° C. Females appeared to acclimate more than males, even proportionally. This can be seen from the changes

in mean tolerance, especially at 24° C. Furthermore, of the 16 animals (out of 144) recovering within 30 min of the temperature shock or failing to succumb at all, 14 were female and 2 were male. Females also seem to be more subject to environmental influences other than exposure temperatures, as indicated by the variances within treatments. These variances were 159.7 for females and 21.0 for males. The greater variance between females is consistent with their greater response to exposure temperature.

By comparison with the low rates of recovery in animals raised at 10° C (above), animals raised at 20° C almost always recovered from the temperature shock (Table II). In this experiment on rearing temperature, progeny from the same stock as above were raised at 20° C and tested after exposure to 10, 18, and 24° C as before. Only males were tested in this case. The results in Table II clearly show the increase in tolerance of the animals raised at 20° C, regardless of

TABLE III

Increased high temperature tolerance of animals raised at 10° C and exposed to 18° C and 23° C for 3 hr and 20 hr. Body of table gives tolerances measured by the shock-recovery assay.

		Mean temperature tolerance	
		Exposure time	
		3 hr	20 hr
Exposure temperature			
10° C (control)	♀	10.0	12.0
	♂	9.0	13.0
18° C	♀	11.5	20.5
	♂	9.5	14.0
23°C	♀	15.0	51.0
	♂	15.5	17.0
		4 animals per mean	4 animals per mean

	Variance analyses for each sex and exposure time			
	Mean squares			
	♀ at 3 hr	♂ at 3 hr	♀ at 20 hr	♂ at 20 hr
Exposure temperatures				
23° C, 18° C *vs.* 10° C	28.2	32.7	1504.2**	10.7
23° C *vs.* 18° C	24.5	72.0*	1860.5**	18.0
Within subclass	7.9	11.8	83.5	63.9
Total variances	11.2	19.2	374.2	54.9

* $P < 0.05$.
** $P < 0.01$.

TABLE IV

Increased high temperature tolerance at higher salinities following acclimation at two temperatures and two salinities for 24 hr. Body of table gives tolerances measured by shock-recovery assay. Animals in Experiment C were shocked at 33.5° C; the others at 34.5° C.

Mean temperature tolerances

	Experiment								
	A			B			C		
	Exposure Temp.	Salinity		Exposure Temp.	Salinity		Exposure Temp.	Salinity	
		0‰	13‰		0‰	13‰		0‰	13‰
♀	20° C	14.8	30.3	13° C	12.3	27.8	11° C	14.4	18.4
♂		11.0	23.8		8.3	12.3		5.8	9.4
♀	24° C	19.8	27.8	23° C	13.2	24.2	23° C	22.6	33.0
♂		13.8	18.8		8.0	11.5		11.6	26.2
	4 animals per mean			6 animals per mean			5 animals per mean		

Variance analyses for each sex

	Mean squares					
	Experiment					
	A		B		C	
	♀	♂	♀	♂	♀	♂
Between temperatures	6.3	5.0	12.0	2.0	649.8*	638.4*
Between salinities	552.3**	315.1**	1053.4**	84.4	259.2	414.1*
Interaction	56.3	60.1	30.4	0.4	51.2	151.3
Within subclass	58.1	28.0	76.0	32.0	90.0	86.9
Total variance	87.5	47.8	113.7	31.6	126.3	136.5

* $P < 0.05$.
** $P < 0.01$.

what temperature they were exposed to later. Thus, acclimation, and adaptation to an elevated temperature, can occur during development, and the effect of a low temperature during development cannot be completely overcome by subsequent exposure to a higher temperature.

Having shown that acclimation occurred, even during development, the next question was whether a short exposure time would suffice. Table III shows that as little as 3 hr at 23° C and certainly less than 24 hr were required for acclimation to occur. There is some evidence that males acclimate earlier and less than do

females. This was indicated also in the earlier data in Table I. The same animals were tested each time, and the correlation between measured tolerances was 0.48 ($P < 0.01$).

All the experiments reported so far were done in water with no detectable salinity. Temperature tolerance was shown earlier to increase when animals collected at 0‰ were placed in higher salinity (Bradley, 1975), so it seemed reasonable to test for an effect of salinity on acclimation. The results of three experiments are shown in Table IV. None of the three experiments gave much indication that acclimation was influenced by salinity. In the first two experiments (A and B) the shock temperature was too high; but even when there was sufficient variation in tolerance (C), no evidence of interaction between exposure temperature and salinity was found.

The data in Table IV cannot be directly compared to those in Tables I and II, since the source of the animals differed and the experiments in Table IV were done almost 9 months later. However, tolerances of females were again higher, as were the variances among females within treatment as discussed earlier.

Additional experiments on acclimation to high temperatures were done using two other criteria, survival times at constant high temperatures and times until complete inactivity of animals in slowly increasing temperatures. In the former experiments, females survived longer than males at both 32° C and 33° C, but there was no evidence of acclimation in animals exposed to 25° C for 24 hr. Mean survival times of females ranged from 9.3 to 13.0 hr and of males ranged from 3.7 to 9.5 hr, depending mainly on test temperature.

There was evidence of acclimation, when time to inactivity was the criterion. Animals exposed to 24° C for 24 hr remained active significantly longer (146–151 min) than did animals kept at 15 hr (74–103 min), when tested in a temperature increasing slowly from 31° C. The temperatures at immobilization were 35.3 to 35.7° C and 32.9° C, respectively.

The reasons for the inconsistency between these two experiments is not clear. In the second set of experiments the total time of observation was less than 2.5 hr, allowing a more accurate measurement of longevity. The stress due to temperature probably was greater in the second experiments, perhaps allowing more accurate expression of the effects of acclimation.

Acclimation to decreased temperature also occurs in *Eurytemora affinis,* although less rapidly than to increased temperatures. Two sets of data were obtained, one set from animals tested for tolerance following exposure to 4, 10, and 15° C for 24 hr and a second set from different animals exposed to 4, 10, and 15° C for 60 hr (Table V). There is clear evidence for acclimation to low temperatures, especially after 60 hr. There also seems to be more acclimation in males by 24 hr and more in females by 60 hr, which is consistent with the inference from Tables I and II that males acclimate earlier and less than do females. However, the sexual dimorphism in degree of acclimation was much less for cold tolerance than for heat tolerance. Finally, the four variances (mean squares) within run and exposure temperature in Table V taken as crude measures of physiological variance, are consistent with the more immediate and smaller flexibility of males.

TABLE V

Increased low temperature tolerance of animals raised at 15° C and exposed to 10° C and 4° C; one set exposed for 24 hr and another for 60 hr. Indices of tolerance are in body of table.

Mean temperature tolerance

		Exposure time	
		24 hr	60 hr
Exposure temperature			
15° C (control)	♀	23.6	31.8
	♂	20.9	22.8
10° C	♀	24.6	35.4
	♂	27.3	23.8
4° C	♀	28.3	50.0
	♂	29.2	35.3
		16 per mean	16 per mean

Variance analyses for each sex

	Mean squares			
	24 hr		60 hr exposure	
	♀	♂	♀	♂
Exposure temperatures				
4° C *vs.* 10° C, 18° C	187.1*	276.8*	3381.4**	1928.0**
10° C *vs.* 15° C	8.0	331.6*	207.1	9.1
Runs	2084.8**	522.4**	137.1	775.1**
Runs × temps.	349.9**	121.5*	231.4	125.2
Within subclass	29.5	51.3	138.6	75.8
Total variance	204.4	101.1	207.2	160.2

* $P < 0.05$.
** $P < 0.01$.

One question raised by these results is whether hot and cold tolerances are similar or different characters. Earlier indications (Bradley, 1975) were that animals resistant to high temperatures tended to be resistant to cold temperatures. To test the relationship more formally, tests of heat and cold tolerances were done twice on 24 animals on successive days. All the correlations except one were positive, eight of twelve were significant. Test-retest correlations (heat-heat, cold-cold) averaged 0.45 for males and 0.70 for females, which were only slightly higher than the average correlations between cold and heat tolerances (0.32 and 0.54, respectively). Hence, there is no evidence that tolerance was biased in one direction in each animal. In other experiments where both tolerances were measured,

correlations were sometimes low but were never negative when averaged over the experiment.

Discussion

Acclimation to high temperatures occurs probably quite quickly (<24 hr), being completed in a few days, perhaps more slowly and certainly more extensively in females than in males. Exposure to increased temperature during development also leads to increased tolerance in adults, beyond what could be achieved by exposure beginning at the adult stage. Apparently, changes affecting temperature tolerance occur during development and are only partially reversible in the adult stage. Although salinity affects temperature tolerance, there is no evidence that acclimation is greater at higher salinity. Acclimation to low temperatures also occurs, but less rapidly than to high temperatures. Effects of sex on acclimation to cold temperatures are also less marked.

The results on acclimation to high temperatures agree with the observations of others. Levins (1969) found that most of the thermal acclimation of *Drosophila* species took place in the first 12 hr. Bowler (1963a) also found that acclimation in the crayfish, *Astacus pallipes*, occurred rapidly and was completed in about two days. Vernberg and Moreira (1974) reported that males of the copepod species *Euterpina acutifrons* had a lower metabolic rate at 15° C than females when both had been acclimated at 25° C. However, males were smaller and the metabolic (respiration) rates were not adjusted for body size. According to the data of McLeese (1956), from a study of the effects of salinity, acclimation, and oxygen tension on lobster survival, there appeared to be little effect of salinity on acclimation.

Data in this study also suggest that acclimation (at least to high temperature) is more easily detected using coma tolerance rather than survival as the criterion. Survival time was not increased following exposure to 25° C, compared with 15° C. Where coma tolerance was the criterion, whether in a shock temperature (Tables I–IV) or in slowly increasing temperature, the data indicate that significant acclimation did take place. Heinle (1969) also reported that thermal tolerances of *E. affinis,* measured as survival in constant environments, was not increased in animals exposed to 20 or 25° C, compared with animals exposed to 10 or 15° C. Hamby (1975) found that acclimation of a marine snail, *Littorina littorea,* significantly shifted the temperature at which the animal entered heat coma but affected the lethal temperature very little. He concluded that the nervous system of *Littorina* was most vulnerable to thermal extremes, as is the case with other poikilotherms (Prosser, 1973).

The influence of acclimation on the nervous system (and so on coma tolerance) is indicated by the results of Baldwin and Hochachka (1970) who showed that different variants of acetylcholinesterase were present in the brains of trout acclimated to different temperatures. Other reported responses to exposure to higher temperature were lowered temperature-specific respiration rates in a toad (Fitzpatrick and Atebara, 1974), lower temperature-specific respiration rates and heart rates in limpets (Markel, 1974), and alterations in enzyme systems donating energy required in the functions of tissue "cation pumps" (Bowler, 1963b).

Having noted the agreement with other results and described possible mechanisms, the question remaining is how such ability to acclimate (individual flexibility) is maintained (or how it arose), when no individual copepods are exposed to the whole range of temperatures in the Chesapeake Bay (0 to 30° C). Daily fluctuations in temperature, together with diurnal migration may be sufficient for physiological flexibility to be an important trait, which is maintained by natural selection. Another (complementary) hypothesis is that tolerances to high and low temperatures are much the same trait genetically. They appear to be related phenotypically, as shown previously (Bradley, 1975, 1976) and reported again in this paper. Thus, the flexibility observed may be the result of natural selection for tolerance to extremes. Such selection would be relaxed in intermediate temperatures, but never reversed. One problem with this explanation is that in several experiments large additive genetic components of variance in temperature tolerance have been observed, which should not be the case if selection is always in the same direction (Bradley, 1978).

Even if there were a single explanation for the flexibility observed, the reasons for the greater flexibilities or acclimation in females are not at all obvious. Female specimens of *Eurytemora* do not store sperm much beyond the first egg sac (Heinle and Flemer, 1975), although such storage may occur occasionally. If males are required for each mating, and there is only a short interval between fertilization and hatching, there is no obvious reason why males should be less tolerant and less flexible than females at high temperatures.

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Summary

The copepod, *Eurytemora affinis,* was tested for its ability to recover from short exposures to a high temperature (temperature tolerance). Animals kept at a warm temperature for several hours or days before the test increasd in tolerance (acclimation). Females showed higher tolerance and acclimation than males. Temperature tolerance was greater at a higher salinity (13‰ *vs.* 0‰), but acclimation was not. Analogous tests were done at low temperatures. Acclimation to cold temperature also occurred, but more slowly. Sexual differences were less marked than for heat tolerance. When tested on the same animals, heat and cold tolerances seemed to be positively related traits.

LITERATURE CITED

Baldwin, J., and P. W. Hochachka, 1970. Functional significance of isoenzymes in thermal acclimation. Acetylcholinesterase from trout brain. *Biochem. J.*, **116**: 883–887.

Bowler, K., 1963a. A study of factors involved in acclimatization to temperature and death at high temperatures in *Astacus pallipes* 1. Experiments on intact animals. *J. Cell Comp. Physiol.*, **62**: 119–132.

Bowler, K., 1963b. A study of factors involved in acclimatization to temperature and death at high temperatures in *Astacus pallipes* 2. Experiments at the tissue level. *J. Cell Comp. Physiol.*, **62**: 133–146.

Bradley, B. P., 1975. The anomalous influence of salinity on temperature tolerances of summer and winter populations of the copepod *Eurytemora affinis*. *Biol. Bull.*, **148**: 26–34.

Bradley, B. P., 1976. The measurement of temperature tolerance: verification of an index. *Limnol. Oceanogr.*, **21**: 596–599.

Bradley, B. P., 1978. Genetic and physiological adaptation of the copepod *Eurythemora affinis* to seasonal temperatures. *Genetics*, in press.

Fitzpatrick, L. C., and M. Y. Atebara, 1974. Effects of acclimation to seasonal temperatures on energy metabolism in the toad *Bufo woodhousei*. *Physiol. Zool.*, **47**: 119–129.

Hamby, R. J., 1975. Heat effects on a marine snail. *Biol. Bull.*, **149**: 331–347.

Heinle, D. R., 1969. Temperature and zooplankton. *Chesapeake Sci.*, **10**: 186–209.

Heinle, D. R., and D. A. Flemer, 1975. Carbon requirements of a population of the estuarine copepod, *Eurytemora affinis*. *Mar. Biol.*, **31**: 235–247.

Levins, R., 1969. Thermal acclimation and heat resistance in *Drosophila* species. *Am. Nat.*, **103**: 483–499.

Markel, R. P., 1974. Aspects of the physiology of temperature acclimation in the limpet *Acmaea limatula* Carpenter (1964): an integrated field and laboratory study. *Physiol. Zool.*, **47**: 99–109.

McLeese, D. W., 1956. Effects of temperature, salinity, and oxygen on the survival of the American lobster. *J. Fish. Res. Board Can.*, **13**: 247–272.

Prosser, C. L., 1973. *Comparative animal physiology, 3rd. ed.* W. B. Saunders, Co., Philadelphia, 966 pp.

Slobodkin, L. B., and A. Rapoport, 1974. An optimal strategy of evolution. *Q. Rev. Biol.*, **49**: 181–199.

Vernberg, W. B., and G. S. Moreira, 1974. Metabolic-temperature responses from the copepod *Euterpina acutifrons* (Dana) from Brazil. *Comp. Biochem. Physiol.*, **49A**: 757–761.